# Adaptive habitat selection by a single celled alga?

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## Summary

1) When populations have opportunity to occupy multiple habitats, individuals that move to habitats with higher mean fitness will expect to produce more descendants than will individuals that are incapable of adaptive habitat choice. Although adaptive movement is widely assumed, we lack understanding of how widely it might apply to motile but non-sentient organisms.
2) I used replicate populations of the single-celled alga, *Chlamydomonas reinhardtii*, to assess its ability to preferentially occupy habitat yielding the highest fitness. I pipetted different densities of *C. reinhardtii* into pairs of shaded and unshaded control Petri dishes filled with growth media. I estimated fitness of this 'clonal' species as per capita growth rate (the ratio of cell densities measured at time $t + 1$ divided by the density at time $t$). I used the estimates to predict the ideal-free distribution of cells expected in adjacent pairs of the two habitats.
3) I created pairs of adjacent shaded and unshaded habitats within two other sets of Petri dishes by covering one-half of each dish with black micromesh. One set of dishes contained unused media, the other set contained the same media in which the cells had been growing (used media). I pipetted algae into either half of these Petri dishes and let cells distribute between habitats for 12 h. I isolated the two halves of each dish and sampled the density of cells occupying each side. I compared the observed distribution with that predicted to test for an ideal-free distribution and calculated fitness to assess adaptive movement.
4) Fitness declined linearly with increasing density in both the light and shade controls, and was higher in light than in shade. When pipetted into the light side of dishes with unused

media, cells were more abundant in light than in shade, and there was no difference in fitness. But when pipetted into the shaded side in dishes with unused media, and in all treatments with used media, there was no significant difference in cell density between habitats even though fitness was usually higher in the light habitat.

5) It thus appears that the ability of *Chlamydomonas reinhardtii* to achieve an ideal free distribution, and more generally to move adaptively, is contingent not only on differences between habitats, but also on the mean quality of the environment in which habitat selection occurs. Regardless, the experiments demonstrate that a motile non-sentient species with simple sensory abilities is clearly capable of adaptive movement that enhances fitness.

## Lay Summary

Faculty and students in the Department of Biology are bound together by a common interest in explaining the diversity of life, the fit between form and function, and the distribution and abundance of organisms. The research reported here shows how habitat selection and adaptive movement influence population dynamics. I first demonstrate by theory why habitat selection should vary with density. I then describe experiments in which I measured per capita population growth rates of the single-celled alga, *Chlamydomonas reinhardtii*, in separate shaded and unshaded (light) habitats across a range of densities, and used them to predict the number of cells that one should observe in adjacent habitats. Fitness was higher in the light habitat than in the shade. Using these observations, theory predicts that all individuals should occupy the light habitat at low density, but as density increases individuals should increasingly occupy the shade. More generally, individual cells should move to habitats of higher mean fitness (adaptive movement). My experiments on *Chlamydomonas* demonstrated a rather novel form of adaptive movement that only partially confirmed the predictions. The fitness of cells released in the light habitat in rich environments was not different from that in shade because density was higher in light. Cells released in shade, and those released in poor environments, moved such that there were no differences in density between habitats, even though fitness was usually higher in light. Adaptive movement thus depends not only on the quality of the occupied habitat, but also on mean environmental quality.

## Acknowledgments

I am grateful for the continued support and guidance for this work from the curious and dedicated nature of my supervisor Dr. Douglas Morris. I thank M. Maki and S. Schroeder for their assistance with laboratory research, and A. Dupuch, W. Halliday, S. Vijayajan, and R. Buchkowski as members of the evolutionary ecology research team. I am also grateful to S. Hecnar, W. Qin, and B. Danielson for insightful advice and comments that helped enhance the value of this thesis. I thank Canada's Natural Sciences and Engineering Research Council (NSERC) for its continuing support of D. Morris' research program in evolutionary ecology. I acknowledge Lakehead University for additional scholarship support and collaborative efforts with Dr. W. Qin from the Biorefining Research Institute. Lastly, I thank my family and friends for encouraging me to pursue this research.

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# Introduction

The vast majority of organisms compete for resources in spatially and temporally stochastic environments where density-dependent habitat selection can modulate population dynamics, species-interactions, and community structure (Rosenzweig 1981; Morris 2011). When organisms live in heterogeneous environments, those individuals which remain in or move to areas of high fitness will expect to produce more descendants than will individuals lacking such adaptive potential (Holt 1985; Abrams 2000). Classical theory imagines that the movement of individuals among habitats that differ in suitability should equalize mean fitness (an ideal free distribution [IFD]; Fretwell & Lucas 1969). The IFD assumes, however, that organisms possess perfect information and move only to increase fitness (Milinski & Parker 1991; Hugie & Grand 1998). Somewhat less 'perfect' organisms are nevertheless likely to move to, or remain in, areas of higher fitness (adaptive movement: Abrams, Cressman & Křivan 2010) but not necessarily equalize mean fitness amongst habitats (Cressman & Křivan 2012).

Experiments that match variation in habitat quality with the sensory capabilities and motility of organisms should be able to detect adaptive movement. Such experiments will be most effective if they can be replicated under strictly controlled conditions and if they can independently assess fitness and density. Thus, I ask whether *Chlamydomonas reinhardtii* (Fig. 1), a motile single-celled alga with phototactic and chemotactic abilities (Harris et al. 2009), can achieve adaptive movement.

I begin by describing how I manipulated population densities of *C. reinhardtii* in order to obtain replicated estimates of fitness (per capita population growth rates) in shaded and unshaded (light) habitats. I use the relationships between fitness and density to predict the expected

distributions of cells in the two habitats assuming ideal-free habitat selection. I then describe experiments where I covered one-half of a set of Petri dishes with micromesh to create adjacent pairs of shaded and unshaded habitats, and assess whether the algae preferentially occupied the habitat yielding the highest fitness. I contrast the observed patterns of distribution and fitness with those predicted from theory and conclude by discussing the evidence supporting adaptive movement, and how it might be constrained in environments of low mean quality.

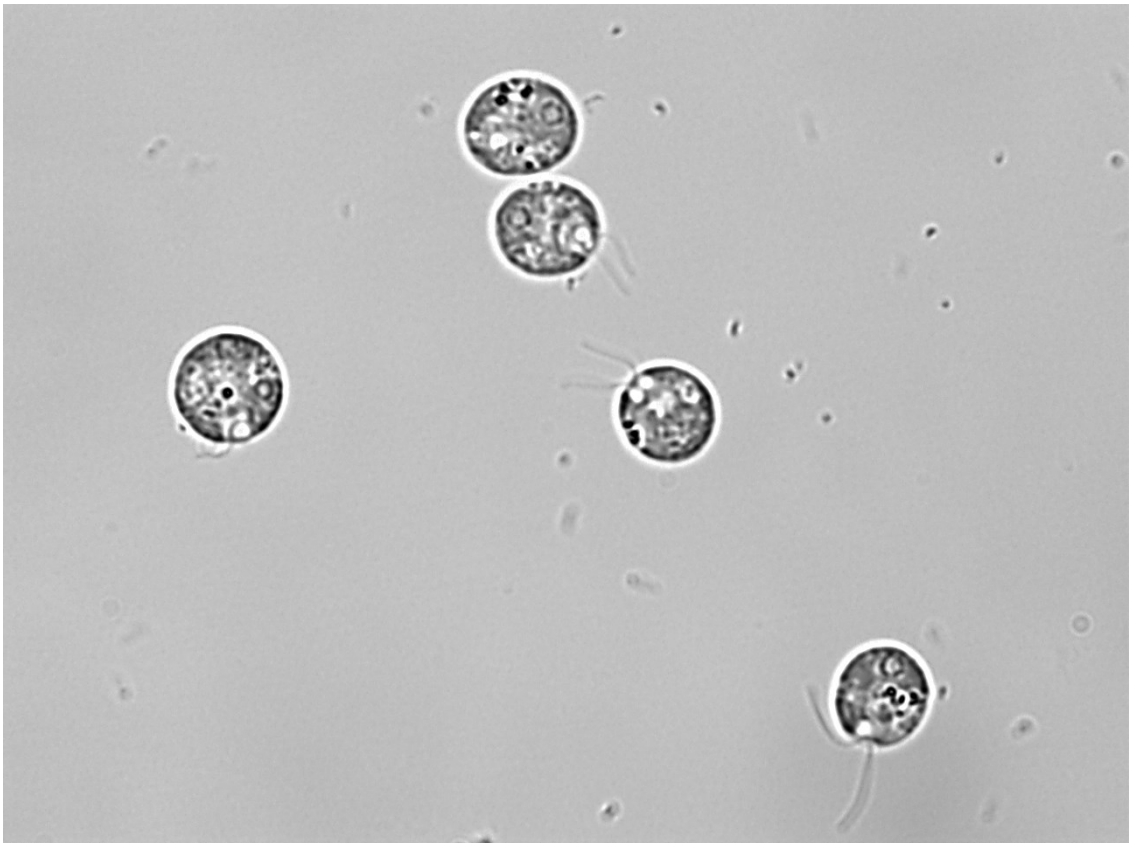
## Materials and methods

### ALGAL CULTURES

Pure batch cultures of wild-type bi-flagellate *Chlamydomonas reinhardtii* (strain CC-2935, *Chlamydomonas* Center, Duke University, NC) were grown anexically in Erlenmeyer flasks containing modified Bold's basal medium (Appendix 3; Bold, 1940; Bell, 1990). Cultures were gently aerated with sterile filtered air at ambient $CO_2$ levels (AIRPUMP 702A, Rena®) using 5-ml glass Pasteur pipettes and flexible plastic tubing (C-Flex tubing, No. 06422-07, Cole Parmer). All cultures were grown synchronously in a growth chamber (ThermoScientific, Model no. 845, CA. USA) set on a 12 h light-dark cycle maintained at 23°C (±1.0°C). Mean (and standard deviation) light intensity obtained from measurements (Amprobe LM631A, WA. USA) taken every five minutes at 12 positions in the growth chamber over one hour (repeated four times at 3-h intervals, $n = 144$,) was 2120 (±160) *lux*.

Cultures were started from single colonies grown on routinely transferred agar plates as described by Harris et al., (2009). Starting cultures were grown in 75 ml of fresh media in 250-ml Erlenmeyer flasks until they reached mid-log-phase density (1-5 million cells·ml$^{-1}$) after five to seven days (Appendix 5; Table A2, p. 54). Aliquots (25 ml) were transferred into 300 ml of

Figure 1: Digitally photographed *Chlamydomonas reinhardtii* cells [CC-2935, wildtype (-)] under 60X magnification using a phase-contrast setting on an inverted microscope (Olympus IX51, USA) in the Lakehead University Instrumentation Lab, July 2010.



media in one litre Erlenmeyer flasks for an additional growth period (7-10 d). Culture densities were adjusted by dilution to achieve randomly allocated target densities, then centrifuged (Sorvall RC 6 Plus, No. 46910, Thermo Scientific, USA) to pelletize the cells (Harris et al. 2009). Cell pellets were washed and re-suspended in 100 ml of fresh media in 500-ml Erlenmeyer flasks, then acclimated for a further 36 h before experimental use (as recommended by Harris et al. 2009).

Optical densities were estimated from spectrophotometer absorbancy readings at 665 *nm* on a microplate spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer #168-1150, Bio-Rad, USA) calibrated with one blank well containing pure media on each microplate. Optical densities of diluted cell cultures were converted to cell densities (millions of cells$\cdot$ml$^{-1}$) with calibration curves from haemocytometer cell counts of immobilized samples (Appendix 4, pp. 47-51; Fig. A7, p. 61).

## EXPERIMENTAL DESIGN

Experiments were conducted in glass Petri dishes (100 x 15 mm, No. 89000-304, VWR® International). All Petri dishes were placed inside 10-cm tall black cardstock cylinders to ensure that cultures received uniform light intensity from the bank of growth lights overhead. Removable partitions made of wax-based modelling clay (NDC57, Polyform Products Co. Illinois, USA) were placed across the middle of each Petri dish to create two equal-sized halves (habitats). Habitats designated as 'shaded' were overlaid with double-layered black fibreglass micromesh (mesh size 0.25 mm$^2$, FCS7350-A, Saint Gobain, CA). The mesh on top of the Petri dish lids reduced the mean (and standard deviation) light intensity from 2120 (±160) to 240

(±35) *lux* (*n* = 144). Unshaded Petri dishes (light habitats) were fully exposed to the mean light intensity of 2120 (±160) *lux*.

Fifteen ml of *C. reinhardtii* culture was pipetted into one-half (the initial half) of the partitioned Petri dishes (15 ml of unused or used media without algae was pipetted into the alternate half). Partitions were removed and cells were free to move throughout the Petri dish for one full photoperiod (12 h). Two controls and two treatments, consisting of three different densities (12 Petri dishes), were tested simultaneously (Figure A5, p. 57). 'Light' controls were unshaded; shade controls were fully covered with mesh. One-half of each dish was covered by mesh in the two habitat-selection treatments. One treatment consisted of cell-culture pipetted into light halves of the three replicate dishes, the other consisted of cell-culture pipetted into shaded halves. All cell-culture transfers, positions in the growth chamber, and the order of processing samples were determined randomly in advance using a random number generator (R Core Development Team 2008).

## FITNESS AND HABITAT SELECTION

Cell densities in each habitat were quantified at time zero, and at 12, 24, and 48 hours after the start of each experiment. At the end of the first photoperiod (12 h), after cells had opportunity to select between habitats, new partitions were inserted between the habitats and remained in place for the duration of the experiment (Appendix 5; Fig. A6, p. 59). Each half of every Petri dish was gently aerated at each sampling interval to ensure accurate and repeatable density estimates within habitats. Eight-220 µl samples from each half were drawn with an eight-tip micropipette oriented parallel to, and equidistant from (225 mm), the partition. All samples were then

pipetted into 96-well microplates (DL-3571172, BD Falcon, CA, USA) and the mean of three optical densities (absorbancy) recorded at 665 *nm* on the spectrophotometer. One 'blank' well containing pure media was used to calibrate the readings obtained from each microplate. Densities recorded at 12 h ($N_{12}$) were used to determine the distribution achieved by habitat selection. Cells divide only during the dark part of the cycle (Harris et al. 2009), so I estimated fitness as the per capita population growth rate achieved between 24 and 48 h (density at 48 h [$N_{48}$] divided by the density at 24 h [$N_{24}$]). Use of the 24-48 h time period guarantees that the growth estimates represent fitness achieved after the cells had completed any habitat choice (division between h 12 and 24 would include fitness obtained by cells that occupied both habitats during the previous photosynthesis and habitat-selection phase of the experiment).

I conducted two sequential sets of experiments using two different kinds of media in order to assess the role of mean environmental quality on habitat selection. I used freshly prepared (unused) media with a full suite of nutrients in the first set of experiments. In the second set, I tested used media (media remaining after I removed cells by centrifugation) from cultures grown at equivalent densities (and for equal durations) as in the experimental cultures. I tested 15 experimental densities in each set of experiments (and for both the shaded and unshaded controls, as well as the two treatments; 120 Petri dishes yielding 240 estimates of density and fitness).

## PREDICTIONS AND TESTS

I designed my experiments to assess whether motile organisms with sensory capability can use those abilities to remain in, or move to, habitats with higher mean fitness (adaptive movement).

I reasoned that an absence of covarying densities between the habitats would rule out density-dependent habitat selection. If the density in one habitat depended on that in the other, if the pattern of density mirrored that predicted from the control habitats, and if there was no difference in mean fitness between them, then I can conclude that *Chlamydomonas* habitat selection achieved an IFD. But if density in one habitat depended on that in the other, if mean fitness was higher in the rich (light) habitat, and if cells preferentially moved to or remained in the light, then I can conclude that *Chlamydomonas* is capable of adaptive habitat selection, but incapable of achieving an IFD.

I estimated the relationships between per capita population growth rates and cell density by geometric mean regressions in R with the lmodel2 package (2.15.2; R Development Core Team 2008). I used those functions (from the controls only) to predict the densities in each habitat such that mean fitness would be the same in each (the habitat isodars, Morris 1987; 1988; an IFD; Appendices 1 & 2, pp. 38-43). I then contrasted the actual relationships from the habitat-selection treatments with that predicted from the controls. I evaluated the fit of the models to the data by verifying that the distributions of residuals were not different from that expected assuming a normal distribution. I concluded the experiments by using paired t-tests to assess whether fitness was different between the initial and alternate halves of the Petri dishes in each experiment. Significance tests for those comparisons were two-tailed because the IFD predicts equal fitnesses and is silent as to which side of the dishes should attain higher fitness by a non-habitat selecting species.

## DIFFUSION CONTROL

My tests for adaptive habitat selection assume that the light versus shade treatments created rich and poor habitats respectively, and that algal distributions differed from that expected by diffusion of the media between the two halves of the Petri dishes. If the media diffuses more slowly than the time-scale of habitat selection, then habitat-selection for light versus shade might be confounded by differences in nutrient concentrations.

I tested for this possibility by pipetting a 15-ml solution of media coloured with a single concentration of non-reactive dye (10µL of dye in 50 ml of media, Bio-Safe coomaisse blue stain, #101-0786, BioRad, USA) into one half of 12 different Petri dishes. I pipetted 15 ml of standard media into the other half of the dishes. I evaluated the time-dependent pattern of diffusion by extracting eight-220 µl paired samples from each side of 12 replicated Petri dishes at one-hour intervals for 12 hours (eight samples from each side of one dish at each interval, each dish used once only). I pipetted the samples into a 96-well microplate and recorded three absorbancy values at 665 *nm* on the spectrophotometer (this wavelength is close to the comaisse blue absorption maximum of 595*nm*, Syrovy & Hodny 1991). I calculated the mean of the three values and assessed whether there were differences between the two halves of the dish with paired-t tests ($n$ = 8 for each of the 12 tests; Table A3, p. 64). I reasoned that the time when I was unable to detect a difference in absorbancy would correspond with that required for diffusion to 'homogenize' the media.

## Results

UNUSED MEDIA

Per capita population growth rate was higher in the control light than in the control shade. Growth rates declined with population density linearly, and in parallel, in both habitats (equations A and B in Table 1, Fig. 2).

In order to assess whether or not the habitat-selection experiments yielded an IFD, I set equations (A) and (B) equal to one another

$$2.37 - 1.20 \times (Density\ in\ light) = 1.87 - 1.29 \times (Density\ in\ shade) \quad (1)$$

and solved for the habitat isodar as the density of cells occupying the light habitat,

$$Density\ in\ light = 0.42 + 1.08 \times Density\ in\ shade \quad (2)$$

(dashed line, Fig. 3c, d). According to the isodar, if cells select between the two habitats according to an ideal free distribution, then they should occupy only the light habitat at densities below 0.42 million cells·ml$^{-1}$, then become ever more evenly distributed between habitats with increasing population size.

All regressions comparing cell densities between sides and habitats in unused media were statistically significant (Equations C-F; Table 1). Intercepts were not different from zero and slopes were not different from one in all comparisons of density between sides of control dishes (Table 1). Similarly, there was no difference in the mean per capita population growth rates between initial and alternate sides of the control Petri dishes (Table 2). Cells given a choice between the two identical halves of the control dishes followed an ideal free distribution.

When released in light habitat, there was no difference in mean fitness of *Chlamydomonas* between light and shade habitats (Table 2), but the resulting isodar departed from that predicted from the control dishes (intercept not different from zero, slope larger than unity (Fig 3c)). Cells did not achieve an ideal free distribution when released in the shaded half of the habitat-selection dishes. There was no difference in density between habitats (Table 1, Fig 3d) even though fitness was higher in the light habitat (Table 2).

Figure 2: The relationships between per capita population growth rates (fitness) and *Chlamydomonas* cell density (millions of cells·ml$^{-1}$) in the control light (open circles) and control shade (filled circles) habitats with unused media ($n$ = 30 for each regression).

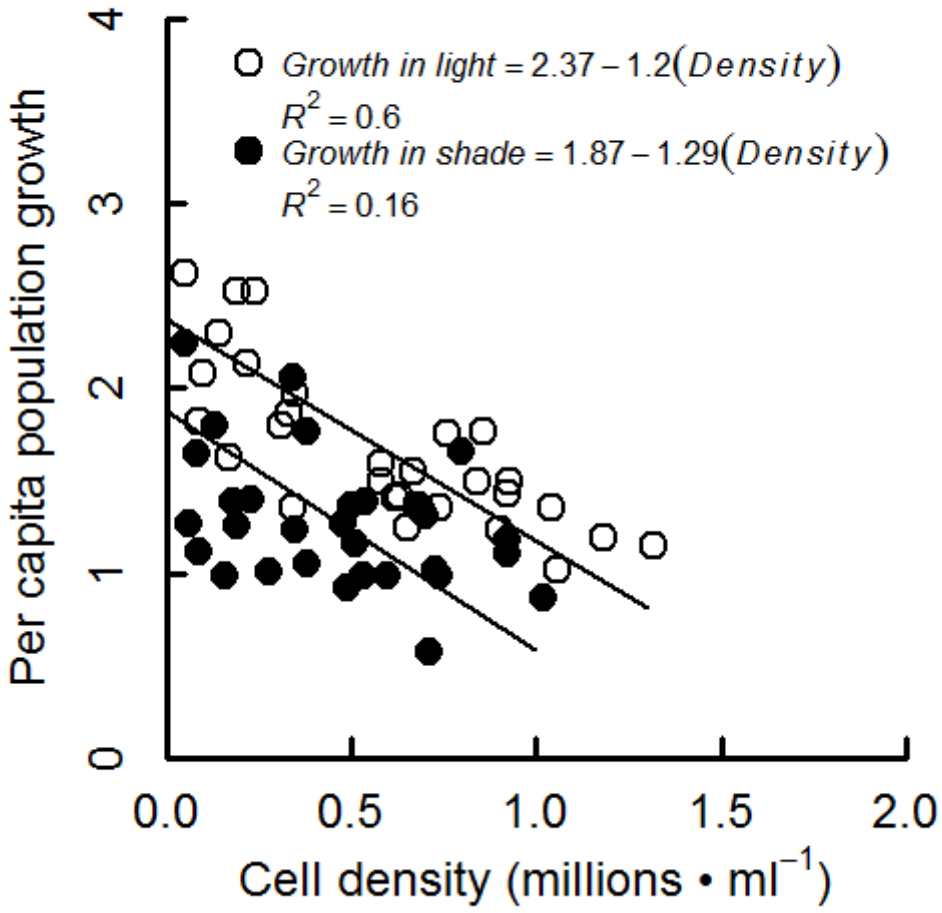

Growth in light = 2.37 – 1.2(Density)
R2 = 0.6
Growth in shade = 1.87 – 1.29(Density)
R2 = 0.16
Per capita population growth
0
1
2
3
4
0.0
0.5
1.0
1.5
2.0
Cell density (millions • ml−1)

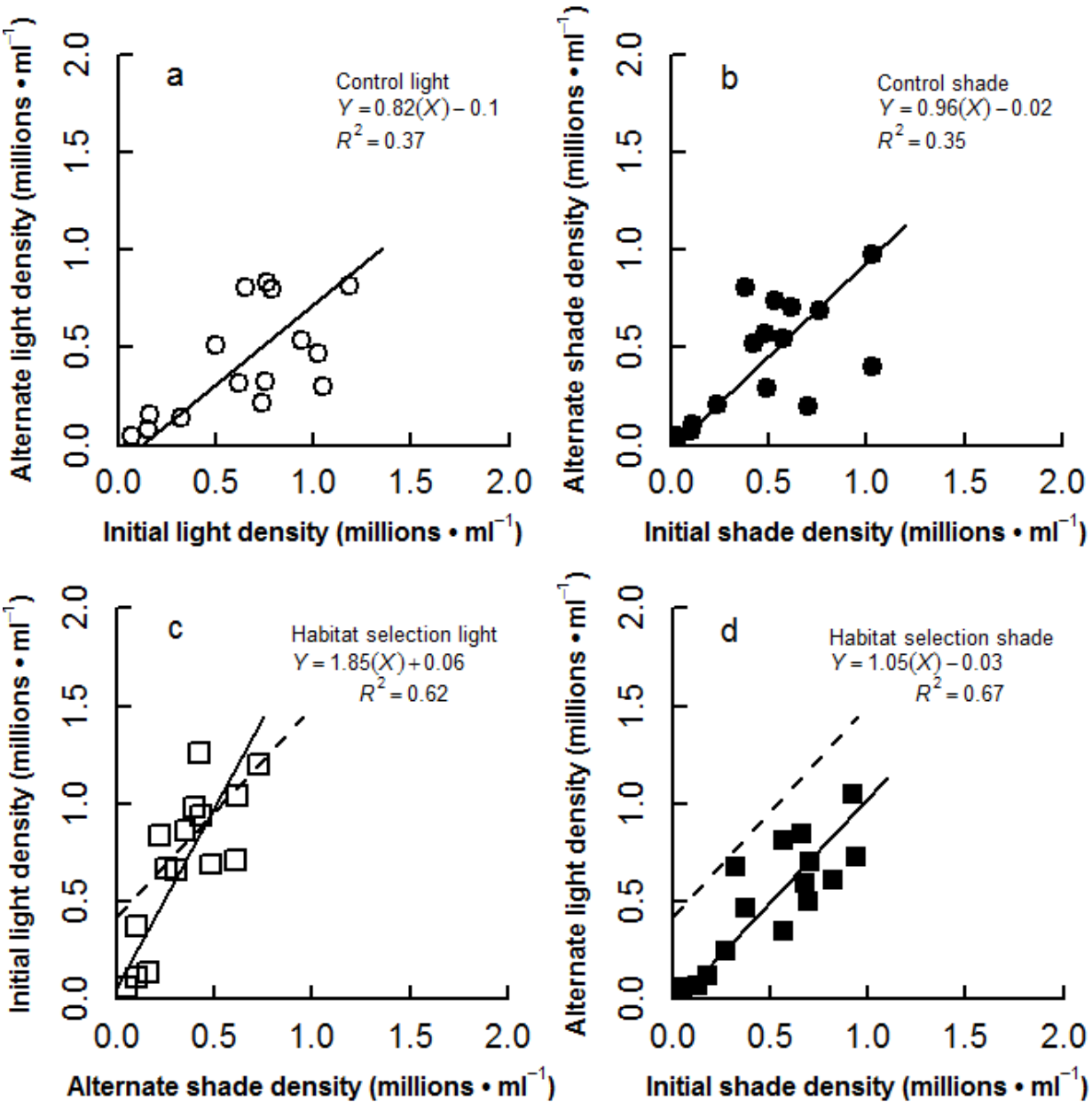
Table 1: Summaries of the relationships between per capita population growth rate (fitness) and cell density, of densities between the two sides of control dishes, and of densities between habitats in treatment dishes for unused media (geometric mean regression; 95% confidence intervals in parentheses). All regressions were statistically significant (bold lettering).

| Comparison | Regression equation | *F* | df | *P* | Eq |
|---|---|---|---|---|---|
| **Per capita population growth rate and density (controls)** | $W_{light} = 2.37\ (\pm 0.2) - 1.20\ (\pm 0.3) \times N_{light}$ | 44.0 | 1, 28 | **<0.001** | A |
| | $W_{shade} = 1.87\ (\pm 0.2) - 1.29\ (\pm 0.4) \times N_{shade}$ | 6.4 | 1, 28 | **0.02** | B |
| **Density between sides (controls)** | $N_{Alternate\ light} = -0.10\ (\pm 0.3) + 0.82\ (\pm 0.4) \times N_{Initial\ light}$ | 9.3 | 1, 13 | **0.01** | C |
| | $N_{Alternate\ shade} = -0.02\ (\pm 0.2) + 0.96\ (\pm 0.4) \times N_{Initial\ shade}$ | 8.7 | 1, 13 | **0.01** | D |
| **Density between habitats** | $N_{Alternate\ shade} = -0.03\ (\pm 0.1) + 0.54\ (\pm 0.2) \times N_{Initial\ light}$ | 21.5 | 1, 13 | **<0.001** | E |
| | $N_{Alternate\ light} = -0.03\ (\pm 0.2) + 1.05\ (\pm 0.4) \times N_{Initial\ shade}$ | 26.6 | 1, 13 | **<0.001** | F |

Table 2: Comparisons of per capita population growth rates between the initial and alternate sides of Petri dishes containing controls and habitat-selection treatments in unused media. Bold lettering identifies statistically significant differences (non-IFD). Paired t-tests; two-tailed significance.

| Side | Habitat | Mean fitness | Paired *T* | *P* |
|---|---|---|---|---|
| Initial | Light | 1.56 | 1.92 | 0.08 |
| Alternate | Light | 1.77 | | |
| Initial | Shade | 1.37 | -1.32 | 0.21 |
| Alternate | Shade | 1.19 | | |
| Initial | Light | 1.55 | -1.8 | 0.09 |
| Alternate | Shade | 1.35 | | |
| Initial | Shade | 1.10 | 3.97 | **0.001** |
| Alternate | Light | 1.79 | | |

Figure 3: The 'isodar graphs' of *C. reinhardtii* (millions of cells·ml$^{-1}$) living in unused media. a and b: habitat isodars comparing initial and alternate sides from control dishes. c and d: regressions of density from the treatment dishes. Open data points represent experiments initiated in the light habitat, filled data points correspond to experiments initiated in the shade. Dashed lines represent the isodar predicted from comparisons between shade and light control dishes.


a
Control light
$Y = 0.82(X) - 0.1$
$R^2 = 0.37$
Alternate light density (millions • ml$^{-1}$)
Initial light density (millions • ml$^{-1}$)
b
Control shade
$Y = 0.96(X) - 0.02$
$R^2 = 0.35$
Alternate shade density (millions • ml$^{-1}$)
Initial shade density (millions • ml$^{-1}$)
c
Habitat selection light
$Y = 1.85(X) + 0.06$
$R^2 = 0.62$
Initial light density (millions • ml$^{-1}$)
Alternate shade density (millions • ml$^{-1}$)
d
Habitat selection shade
$Y = 1.05(X) - 0.03$
$R^2 = 0.67$
Alternate light density (millions • ml$^{-1}$)
Initial shade density (millions • ml$^{-1}$)
0.0
0.5
1.0
1.5
2.0

USED MEDIA

Again, as in unused media, per capita population growth rate was higher in the control light than in the control shade. Growth rates in controls declined with population density linearly, but much more slowly in shade than in light (equations G and H in Table 3, Fig. 4). The maximum per capita population growth in the control light was slightly lower than in unused media, as was the decline with density (Table 3; contrast Fig. 2 with Fig. 4). There was no difference in fitness between paired halves of control dishes in light (Table 4), but the regression comparing paired densities in the two sides of the dishes was not significant (Fig. 5a, no evidence for density-dependent habitat selection, and thus, not an IFD). Fitness and density in the initial shade side exceeded that in the alternate shaded side (Table 4, Fig. 5b). Contrary to unused media, cells in used-media controls failed to achieve an IFD. Nevertheless, I set equations (G) and (H) equal to one another,

$$2.15 - 0.82 \times (Density\ in\ light) = 1.27 - 0.39 \times (Density\ in\ shade) \quad (3)$$

and solved the expected isodar as the cell density in the light habitat,

$$Density\ in\ light = 1.07 + 0.48 \times Density\ in\ shade \quad (4)$$

(dashed line, Fig 5c, d). According to equation (4), if cells select between the two habitats according to an ideal free distribution, then they should occupy only the light habitat at densities below 1.07 million cells·ml$^{-1.}$ Then, for each increase in population size, approximately half as many cells should occupy the lighted half of the dish as occupy the shaded half.

Neither habitat-selection treatment with used media produced patterns in habitat densities different from linear one-to-one relationships with zero intercepts and slopes of unity (Table 3,

Figure 4: The relationships between fitness (per capita population growth rates) and *Chlamydomonas* cell density (millions of cells·ml$^{-1}$) in the control light (open circles) and control shade (filled circles) habitats with used media ($n$ = 26 and $n$ = 29 respectively).

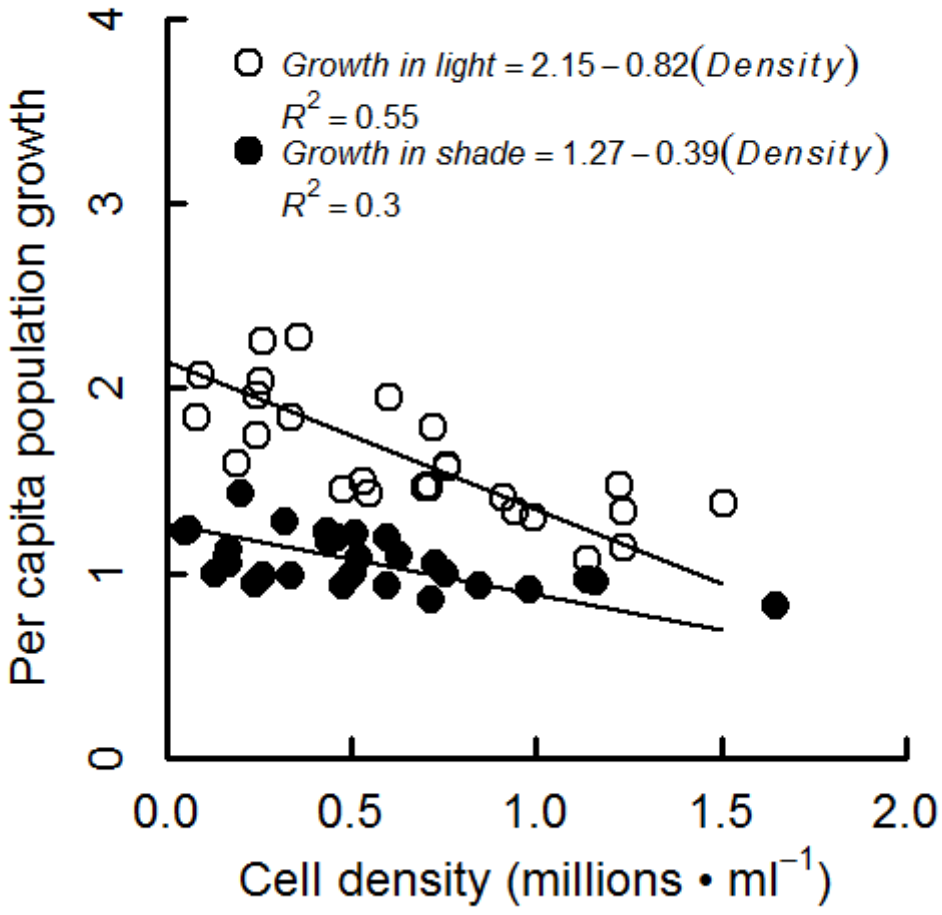

Growth in light = 2.15 – 0.82(Density)
R2 = 0.55
Growth in shade = 1.27 – 0.39(Density)
R2 = 0.3
Per capita population growth
0
1
2
3
4
0.0
0.5
1.0
1.5
2.0
Cell density (millions • ml−1)

Table 3: Summaries of the relationships between per capita population growth rate (fitness) and cell density, of densities between the two sides of control dishes, and of densities between habitats in treatment dishes for used media (geometric mean regression; 95% confidence intervals in parentheses). Bold lettering identifies statistically significant differences.

| Comparison | Regression equation | *F* | df | *P* | Eq |
|---|---|---|---|---|---|
| **Per capita population growth rate and density (controls)** | $W_{light} = 2.15\ (\pm 0.2) - 0.82\ (\pm 0.2) \times N_{light}$ | 32.6 | 1, 25 | **<0.001** | G |
| | $W_{shade} = 1.27\ (\pm 0.1) - 0.39\ (\pm 0.1) \times N_{shade}$ | 13.2 | 1, 27 | **0.001** | H |
| **Density between sides (controls)** | $N_{Alternate\ light} = 0.12\ (\pm 0.3) + 0.67\ (\pm 0.4) \times\ N_{Initial\ light}$ | 3.5 | 1, 13 | 0.08 | I |
| | $N_{Alternate\ shade} = -0.06\ (\pm 0.2) + 1.32\ (\pm 0.4) \times\ N_{Initial\ shade}$ | 9.6 | 1, 13 | **<0.01** | J |
| **Density between habitats** | $N_{Alternate\ shade} = -0.12\ (\pm 0.2) + 0.91\ (\pm 0.4) \times N_{Initial\ light}$ | 14.2 | 1, 13 | **<0.01** | K |
| | $N_{Alternate\ light} = -0.15\ (\pm 0.2) + 0.84\ (\pm 0.4) \times\ N_{Initial\ shade}$ | 7.4 | 1, 13 | **0.02** | L |

Table 4: Comparisons of per capita population growth rates between the initial and alternate sides of Petri dishes containing controls and habitat-selection treatments in used media. Bold lettering identifies statistically significant differences (non-IFD). Paired t-tests; two-tailed significance.

| Side | Habitat | Mean fitness | Paired *T* | *P* |
|---|---|---|---|---|
| Initial | Light | 1.56 | 1.82 | 0.09 |
| Alternate | Light | 1.91 | | |
| Initial | Shade | 1.12 | -2.20 | **0.05** |
| Alternate | Shade | 1.04 | | |
| Initial | Light | 1.81 | -5.01 | **<0.001** |
| Alternate | Shade | 1.04 | | |
| Initial | Shade | 1.12 | 3.97 | **<0.001** |
| Alternate | Light | 2.16 | | |

Figure 5: The 'isodar graphs' of *C. reinhardtii* (millions of cells·ml$^{-1}$) living in used media. a and b: habitat isodars comparing initial and alternate sides of control dishes. c and d: regressions of density from the treatment dishes. Open data points represent experiments initiated in the light habitat, filled data points correspond to experiments initiated in the shade. Dashed lines represent the isodar predicted from comparisons between shade and light control dishes.

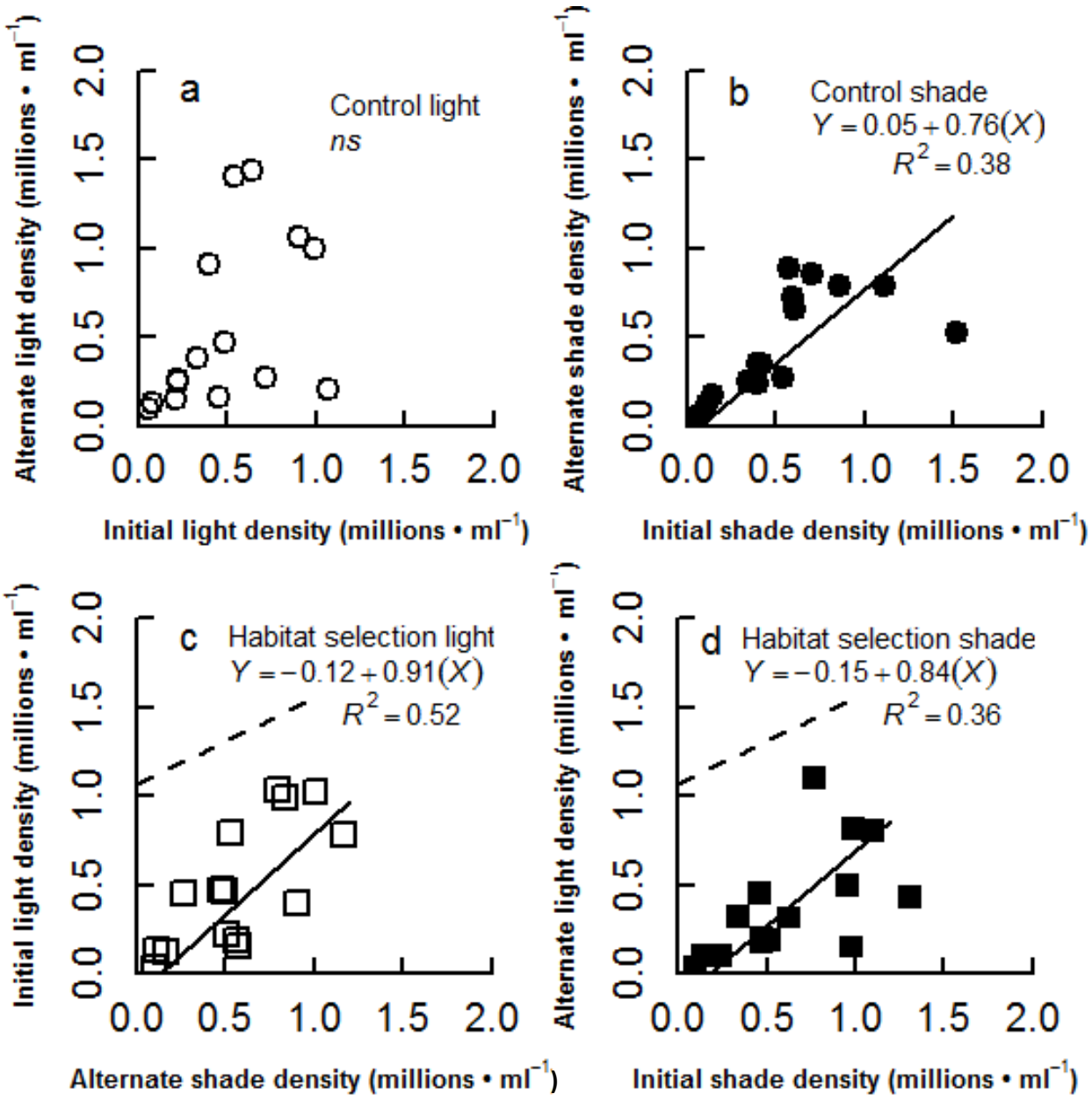



Fig. 5c & d). Per capita population growth rates were significantly greater in light regardless of which habitat the cells were released into (Table 4). Although there was no clear evidence of

differences in density between habitats, the pattern in the residuals suggests a possible preference for the light habitat at low density (Fig. 5c & d). This preference, if real, is consistent with the higher per capita population growth rates observed in that habitat at low density (Fig. 4).

DIFFUSION

Mean absorbancy by dye was dramatically greater on the initial side of the dishes until about hour 4, after which absorbancy was more-or-less homogeneous in both halves of the dishes (Appendix 7, p. 62). It is thus reasonable to assume, for much of the 12-h time course available for habitat selection, 1, that media did not differ between light and shaded habitats, and 2, that differences between habitats were thus caused mainly by differences in light intensity.

## Discussion

Habitat selection operates through the process of adaptive movement such that individuals can increase fitness by moving to a different habitat. Motile organisms should thus evolve sensory capabilities that enable preferential occupation of habitats yielding higher fitness than others. The experiments reported here document that even simple organisms are capable of adaptive movement that yield repeated, but often less than ideal, patterns of habitat selection.

Adaptive habitat selection was best demonstrated in the experiments with unused media. When cells were released in either light or shade controls, there was no difference in density or fitness between the two halves of the dishes. But when cells were released in the light habitat and given the opportunity to move to shade, density was greater in the light habitat even though

480 my diffusion test showed that nutrient concentrations should have equilibrated between habitats.
481 Cells released in light thus attained an ideal free distribution (fitness was not different between
482 habitats), but the densities were not those predicted from the controls.

483 Conversely, there was no detectable difference in density between habitats when cells
484 were exposed to used media, or released in the shade with unused media, even though mean
485 fitness was higher in the light than in the shade in all treatments where cells demonstrated
486 density-dependent habitat selection. How can adaptive movement account for these differing
487 results?

488 Although the experiments maintained constant differences between light and shade
489 habitats in light intensity, cell division was likely limited by local resource depletion as cells
490 metabolized nutrients (Tilman 1990). Thus, diffusion of nutrients from used versus unused
491 media should influence per capita population growth. In rich environments where unused media
492 diffuses between habitats, cells in the light habitat are likely to receive adequate nutrients to
493 maintain high photosynthetic output and reproduction. In poor (used media) environments,
494 however, the availability of nutrients may limit photosynthetic efficiency and cell division such
495 that there is little advantage in choosing one habitat over the other.

496 Regardless, fitness at most densities was higher in light habitat than in shade, and one
497 should expect, *ceteris paribus*, a preference for light by phototactic algae. All is not quite equal,
498 however, because cells living at high light intensities are exposed to increased oxidation of
499 photosystems that require expensive cellular repairs (Harris et al. 2009). It is thus possible that
500 the costs of oxidative stress in light limit the otherwise density-dependent benefits associated
501 with occupying that habitat. Once restricted to the light habitat at the end of the 12-h selection
502 phase, the cells might then invest in physiological adjustments to increase protection and

503 photosynthetic efficiency (Poulin et al. 2009). Those adjustments could then materialize as
504 higher mean fitness (between 24 and 48 h) than the cells initially 'anticipated' during oxidative
505 stress.

506 Some readers might question whether the chemotactic ability of *C. reinhardtii* also
507 accounts for the apparent inability to preferentially occupy the habitat (light) yielding high
508 fitness. I suspect not because diffusion should have equalized nutrient concentrations between
509 habitats long before cells completed habitat selection. It is thus difficult to imagine chemotactic
510 cues that, in my experiments, would consistently 'attract' cells to one habitat or the other.

511 When cells are released in light, the difference in movement responses between used and
512 unused media are consistent with Křivan et al.'s (2008) prediction that the probability of
513 emigration decreases with the increased suitability of the initial habitat. Cells appear to choose
514 one habitat over another only when there is a strong signal of differences in quality between
515 them that can overcome any costs associated with habitat selection. A similar pattern of
516 apparently adaptive 'non-movement' occurs in laboratory populations of rotifers (*Brachionus*
517 *calyciflorus*) which decrease speed and increase turning frequencies when occupying a high
518 quality habitat (Kuefler, Avgar & Fryxell 2013). *C. reinhardtii* appears to possess a movement
519 strategy similar to that of the rotifers. Cells living under diminished light switch stochastically
520 between synchronous and asynchronous flagellar beating patterns that produce intervals of
521 straight swimming with abrupt re-orientations (Poulin et al. 2009). Such simple decision making
522 can likely pay substantial dividends in fitness because the swimming pattern increases the
523 probability of contacting higher-quality habitat. The caveat is that *Chlamydomonas* may only be
524 able to reap those dividends when resources are sufficiently abundant to maximize their
525 photosynthetic capacity.

Algal habitat selection is further complicated by an ability to rapidly acclimate to different light conditions. The adaptive value of emigrating from the shade may thus deteriorate through time because the probability of acclimating to a different light intensity increases with the length of exposure (cells can begin acclimating to different light intensities within minutes, Bonente et al. 2012). The 'doubling' of costs associated with re-acclimating to light should reduce the penchant for movement by cells that have previously acclimated to shade.

The acclimation-cost hypothesis, which has similarities to Stamps' 'silver spoon' and 'natal habitat preference induction' models (Davis & Stamps 2004; Stamps 2006; Stamps, Luttbeg & Krishnan 2009), likely accounts for why my best evidence for an ideal free distribution was associated with control dishes in which I observed no difference between halves in either density or fitness. Cells moving between identical 'habitats' do not change photosystems and glide through the media with normal flagellar movements that are unlikely to entail significant additional costs of habitat selection. Acclimation costs of habitat selection also likely account for the departure of the 'released in light' isodar from that predicted by data from control dishes. Cells migrating to shade must pay the cost of changing photosystems, but cells grown in light controls do not. This important caveat, that controls may not fully account for fitness expectations between habitats, should be carefully contemplated in future tests of habitat selection.

Regardless as to mechanisms, selection of high fitness habitat by *C. reinhardtii* in this study was conditional on whether nutrient concentrations were high (unused media) or low (used media). Although conditional strategies of habitat selection are inferior to density-dependent habitat choice, they can be adaptive when habitats of different quality remain constant over long periods of time (Morris, Diffendorfer, & Lundberg 2004). It will be interesting to learn whether

habitat selection by more sentient organisms also depends on differences between habitats conditioned by the mean quality of the environment.

Adaptive movement emphasizes the benefits of increased fitness as individuals select habitat in response to differences in their environment (Abrams 2000; Cressman & Křivan 2012). The distribution and fitness of *C. reinhardtii* in my experiments documents abilities of adaptive habitat choice originally developed mainly for sentient organisms (Fretwell & Lucas 1969; Flaxman & deRoos 2007). My research demonstrates rather clearly that even so-called simple single-celled organisms are capable of adaptive movements that modify spatial distribution and population dynamics. The biggest surprise, however, is not that algae are capable of adaptive habitat selection, but rather that adaptive movement is so often neglected by ecologists studying the dynamics of populations and communities.

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650 **Appendix 1**: DENSITY-DEPENDENT HABITAT SELECTION

651 Habitat selection refers to the process by which an individual chooses an area in which to
652 conduct specific activities and interact with others (Stamps 2009). Habitat selection emerges
653 because organisms are better suited to live and reproduce in some places than others (Morris et
654 al. 2008). Individuals maximizing fitness should occupy the best habitat available. When
655 increasing density depresses fitness to that of lower-quality habitats, individuals should disperse
656 to those habitats ((Fretwell & Lucas 1969; Rosenzweig 1981; Morris 1987; Johnson & Gaines
657 1990; Holt & Barfield 2001). Thus, if individuals possess 'complete' knowledge of all habitat
658 qualities, are of equal competitive ability, and if there is no cost to movement, then the
659 distribution of individuals among habitats should fit an ideal free distribution (IFD) such that
660 mean fitness is equal in every occupied habitat. If we assume logistic population growth such
661 that

662 $$\frac{dN_i}{dt} = r_i N_i \left[1 - \frac{N_i}{K_i}\right] \quad \text{(A1)}$$

663 where $N_i$ is the population density, $r_i$ is the maximum growth rate, and $K_i$ is the carrying capacity
664 in habitat $i$, and estimate fitness ($W_i$) as per capita growth rate, then

665 $$W_i = \frac{1}{N_i}\frac{dN_i}{dt} = r_i - \frac{r_i N_i}{K_i} \quad \text{(A2)}$$

666 (fitness declines linearly with increasing density, Fig. A1).

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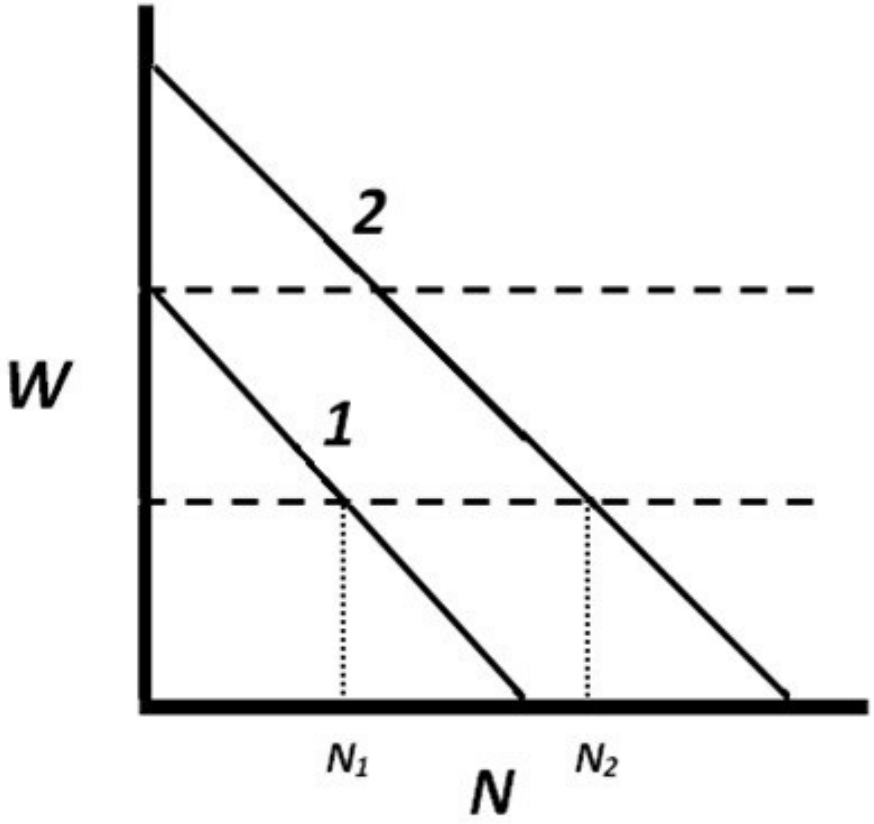
Figure A1: An illustration of ideal-free habitat selection with logistic population growth. Ideal-free habitat selectors achieve equal expectations of fitness ($W$) in habitats 1 and 2 (dashed horizontal lines), but at different population densities ($N_1$ and $N_2$, dotted horizontal lines).


2
W
1
$N_1$
N
$N_2$

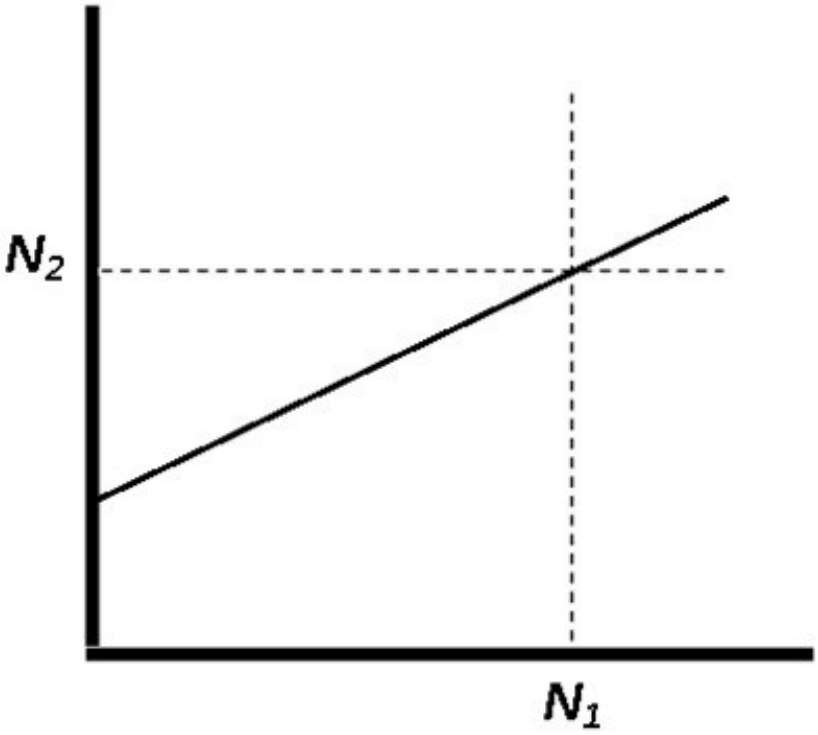
**Appendix 2:** HABITAT ISODAR

Imagining that two habitats differ in logistic population growth, then the solution to ideal habitat selection is given by the habitat isodar (Morris 1987;1988)

$$N_2 = \frac{r_2 - r_1}{r_2} K_2 + \frac{r_1}{r_2}\frac{K_2}{K_1} N_1 \quad \text{(A3)}$$

(Fig. A2; Morris 1987; 1988). If one knows the rate at which fitness declines with density in two or more habitats, as in my experiments with *Chlamydomonas reinhardtii*, one simply needs to set the two fitness functions equal to one another in order to model the IFD isodar *a priori*. Knowing the isodar expected from ideal-free habitat selection enables a rigorous test for the ideal free distribution as long as one can then expose populations to the two habitats experimentally. The similarity between the predicted isodar and the distribution of individuals in the two-habitat experiments will reveal the ability of the organisms to achieve an ideal free distribution.

Figure A2: An example of an ideal-free habitat isodar (the distribution of individuals between two habitats such that mean fitness is equal in each) that emerges when fitness declines linearly with increasing density ($N$) in habitats 1 and 2 as in figure A1. The dashed lines correspond to the carrying capacities in each habitat.


$N_2$
$N_1$

**Appendix 3:** BOLD'S BASAL MEDIUM

I prepared modified Bold's basal medium for standard stock solution in four 1-litre batches (Table A1; Bold 1949; Bell 1990). I added the first six macronutrient salt solutions individually after complete dissolution into the medium, followed by trace metal and nutrient solutions (again, added individually after complete dissolution into the medium). I filtered ferrous sulphate heptahydrate through Whatman's filter paper No. 1, then autoclaved the filtered solution with the rest of the dissolved ingredients before combining them to create the complete medium. I adjusted the pH after autoclaving when necessary [pH = 6.7 (+/-0.2)].

Table A1: Stock solutions and volumes of each compound used in the recipe for modified Bold's basal medium. Original stock solution and refinements are listed as in Bold (1949) and Bell (1990).

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| Chemical compound | 1 Litre Stock Solution | Quantity added to 1 litre of stock medium | Molar Concentration in final medium |
|---|---|---|---|
| **$NaNO_3$** | 25 g $L^{-1}$ $ddH_2O$ | 10 ml | 2.94 x$10^{-3}$ M |
| **$CaCl_2 \cdot 2H_2O$** | 2.5 g $L^{-1}$ $ddH_2O$ | 10 ml | 1.7 x $10^{-4}$ M |
| **$MgSO_4 \cdot 7H_2O$** | 7.5 g $L^{-1}$ $ddH_2O$ | 10 ml | 3.04 x $10^{-4}$ M |
| **$K_2POH_4$** | 7.5 g $L^{-1}$ $ddH_2O$ | 10 ml | 4.31 x $10^{-4}$ M |
| **$KH_2PO_4$** | 17.5 g $L^{-1}$ $ddH_2O$ | 10 ml | 1.29 x $10^{-3}$ M |
| **NaCl** | 2.5 g $L^{-1}$ $ddH_2O$ | 10 ml | 4.28 x $10^{-4}$ M |
| **EDTA anhydrous** | 50 g $L^{-1}$ $ddH_2O$ | 1 ml | 4.28 x $10^{-4}$ M |
| **KOH** | 31 g $L^{-1}$ $ddH_2O$ | 1 ml | 1.38 x $10^{-3}$ M |
| **$FeSO_4 \cdot 7H_2O$** | 4.98 g $L^{-1}$ $ddH_2O$ | 1 ml | 4.48 x $10^{-5}$ M |
| **$H_2SO_4$** | 24.5 g $L^{-1}$ $ddH_2O$ | 1 ml | 1 x $10^{-3}$ M |
| **$H_3BO_3$** | 11.42 g $L^{-1}$ $ddH_2O$ | 1 ml | 4.62 x $10^{-4}$ M |
| **$ZnSO_4 \cdot 7H_2O$** | 8.82 g $L^{-1}$ $ddH_2O$ | 1 ml | 7.67 x $10^{-5}$ M |
| **$MnCl_2 \cdot 4H_2O$** | 1.44 g $L^{-1}$ $ddH_2O$ | 1 ml | 1.82 x $10^{-5}$ M |
| **$MoO_3$** | 0.71 g $L^{-1}$ $ddH_2O$ | 1 ml | 1.23 x $10^{-5}$ M |
| **$CuSO_4 \cdot 5H_2O$** | 1.57 g $L^{-1}$ $ddH_2O$ | 1 ml | 1.57 x $10^{-5}$ M |
| **$Co(NO_3)_2 \cdot 6H_2O$** | 0.49 g $L^{-1}$ $ddH_2O$ | 1 ml | 4.21 x $10^{-6}$ M |

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**Appendix 4:** QUANTIFYING CELL DENSITY

I calculated cell densities (millions of cells·ml$^{-1}$) by fitting optical densities (spectrophotometer absorbancy) to haemocytometer cell counts by geometric mean regression. I used a dilution series to create different densities, then measured absorbancy at 665 *nm* in a microplate spectrophotometer (BioRad xMark™ Microplate Absorbance Spectrophotometer, 168-1150, CA, USA). I immobilized cells from corresponding samples in the two chambers of a Neubauer haemocytometer (Hausser Scientific, Catalogue # 3110, PA, USA) with a drop of Iodine-Potassium-Iodide (Lugol's stain, 6% KI, and 4% I).

I photographed the two chambers in the prepared haemocytometer slides separately with a microscope camera (OptixCam VS1.009, VA, USA), displayed the digital images on a computer monitor (Figure A7), then counted the number of cells in the four corner grids in each chamber's field-of-view. I used a total of 18 separate samples to calibrate optical densities for light and shade habitats at both the start and end (12 h) of the light cycle. Each estimate represents the mean number of cells counted from four separate images covering four separate sections of the haemocytometer grid. Methods for all of the haemocytometer cell counts were from Leboffe & Pierce (2005).

Figure A3: Regressions used to calibrate cell densities (millions of cells·ml$^{-1}$) from optical densities (absorbancy at 665 *nm*) at the start of the light cycle for both light (a: open squares) and shaded (b: filled squares) habitats ($n$ = 18 each). OD = optical density.

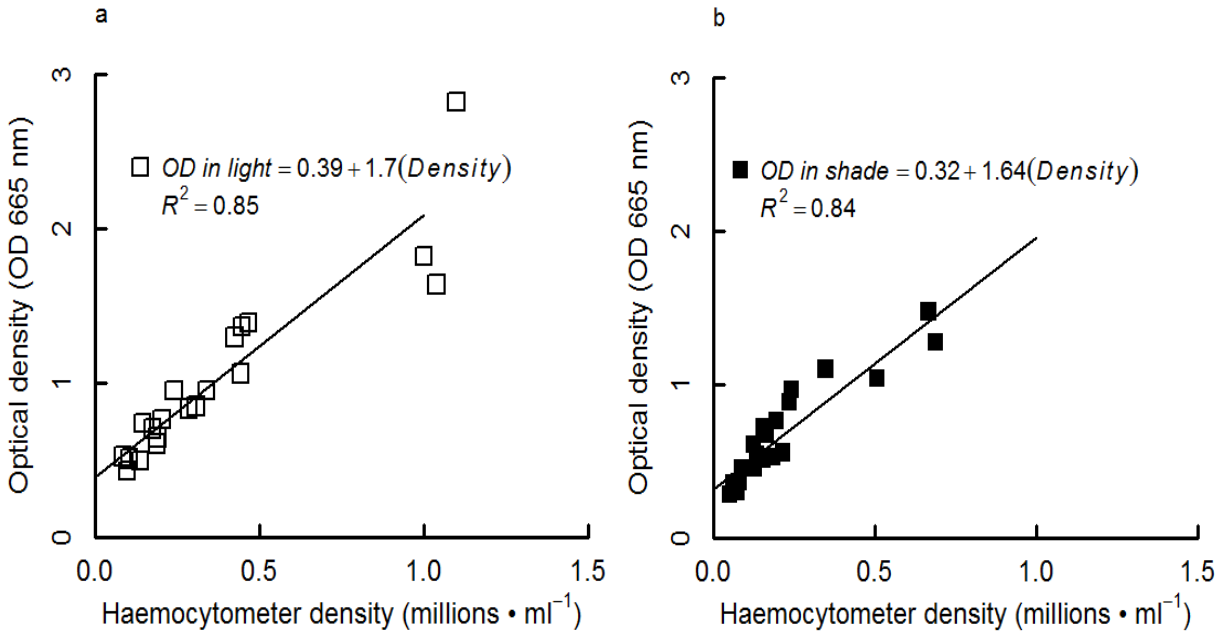
a
b
Optical density (OD 665 nm)
Haemocytometer density (millions • ml$^{-1}$)
OD in light = 0.39 + 1.7(Density)
$R^2$ = 0.85
OD in shade = 0.32 + 1.64(Density)
$R^2$ = 0.84
0
1
2
3
0.0
0.5
1.0
1.5

Figure A4: Regressions used to calibrate cell densities (millions of cells·ml$^{-1}$) from optical densities (absorbancy at 665 *nm*) at the end of the light cycle (12 h) for both light (a: open squares) and shaded (b: filled squares) habitats ($n$ = 18 each). OD = optical density.

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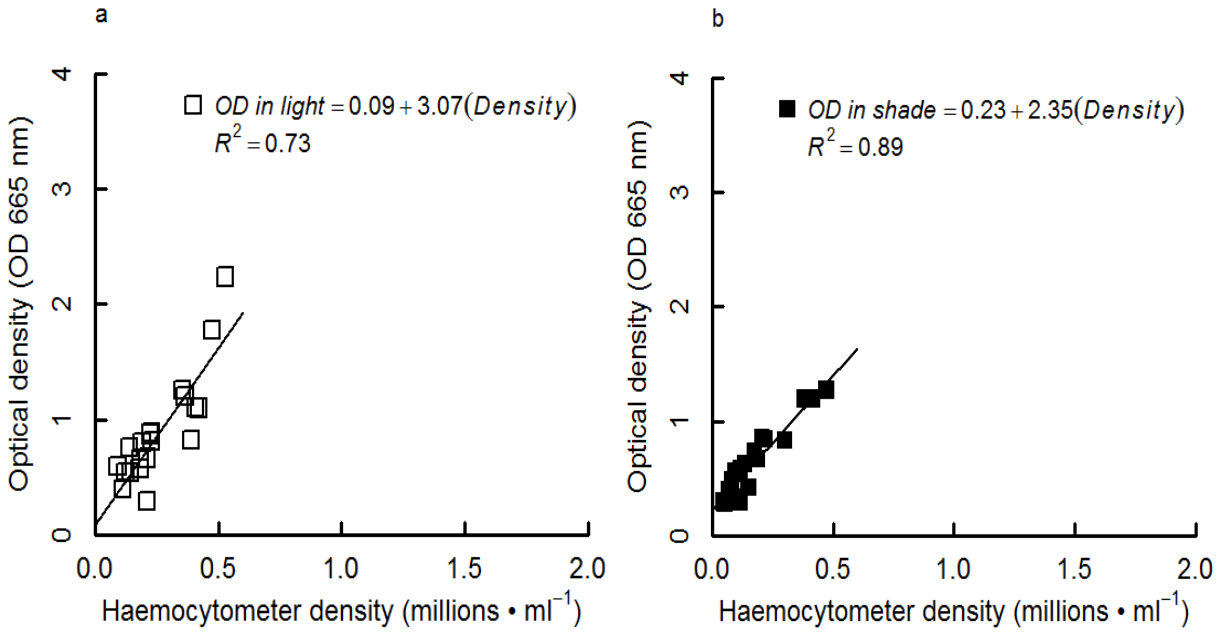


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**Appendix 5:** LABORATORY & EXPERIMENTAL PROTOCOLS

Table A2: Culturing and experimental protocol used to assess habitat selection by *Chlamydomonas reinhardtii*. Experiments conducted between September 2012 and January 2013.

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| Time | Culturing and experimental protocols |
|---|---|
| **Day 0** | Transfer single colony-forming units (CFUs) from enriched agar plates into 75 ml of Bold's medium in 250 ml Erlenmeyer flasks. Grow 'starting' cultures for 5-7 d to a mean density of 2.5 million cells·ml$^{-1}$. Repeat for additional cultures as required. Grow all cultures in a controlled growth chamber at 23ºC, set on a 12 h light:dark cycle to synchronize cellular division (Harris et al. 2009). |
| **Day 6-8** | Pipette 25-ml from each 'starting' culture into 300 ml of fresh medium in one litre Erlenmeyer flask for an additional growth period (7-10 d). Repeat for additional flasks. |
| **Day 14-18** | Adjust densities from batch cultures to three predetermined experimental densities by centrifugation and or dilution. Pelletize experimental cell cultures by centrifugation and re-suspend in 100 ml of fresh medium in 500 ml Erlenmeyer flasks for 36 h before use in order to acclimate cells to experimental conditions (Harris et al. 2009). |
| **Start of Experiment** | |
| **Time Zero (~day 20)** | Pipette 15 ml of experimental culture into one half of each partitioned Petri dish. Repeat with 4 dishes (one for each treatment) at three separate densities to be tested simultaneously. Pipette 15 ml of unused (or used media) medium into the alternate half of each Petri dish. Remove partitions from Petri dishes. Draw 8-220µl aqueous samples from each half of the dishes, parallel to the partition, and transfer into 96-well microplates. Record optical densities from spectrophotometer absorbencies at 665 *nm*. Overlay dishes with two layers of black nylon mesh for shade treatments. Place dishes in the incubator and do not disturb dishes for 12 h. |
| **Time 12** | Remove shade covers and insert new partitions along the central axis of each Petri dish and aerate each culture. Draw 8-220µl aqueous samples from each half of the dishes parallel to the partition, and transfer into 96-well microplates. Record optical densities from spectrophotometer absorbencies at 665 *nm*. Reposition covers for shade treatments. |
| **Time 24** | Repeat sampling. Remove shade cover, then aerate and sample cell densities in each half of each Petri dish. Draw 8-220µl aqueous samples from each half of the dishes perpendicularly to the partition, and transfer into 96-well microplates. Read optical densities on the spectrophotometer at 665 *nm*. Reposition shade cover and then repeat with next dish. |
| **Time 48** | Remove shade covers and insert new partitions along central axis of Petri dish and aerate culture in each half of the dishes. Draw 8-220µl aqueous samples from each half of the dishes perpendicular to the partition, and transfer into 96-well microplates. Record optical densities from spectrophotometer absorbencies at 665 *nm*. |
| **End of experiment** | |

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**Appendix 6:** PHOTOGRAPHS

Figure A5: Photograph illustrating an example of the random placement of control (fully covered by shade and fully exposed to light) and habitat-selection Petri dishes (half shaded) used to assess habitat selection by *Chlamydomonas reinhardtii*. Each of the three replicate dishes contains a different algal density. Image taken at 12 h.

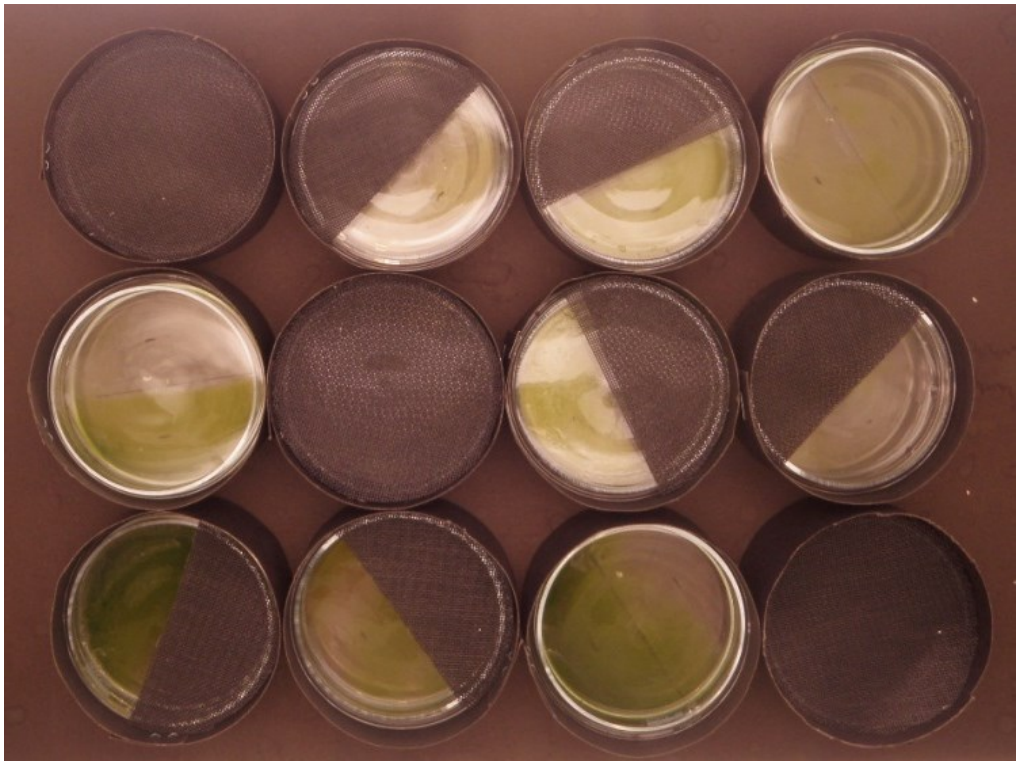


Figure A6: Photograph of a Petri dish illustrating habitat selection by *Chlamydomonas reinhardtii* cells released in the light habitat (right-hand side) with opportunity to occupy shade (unused media). Initial density at time zero = 0.85 million cells·ml$^{-1}$. Image taken at 12 h.

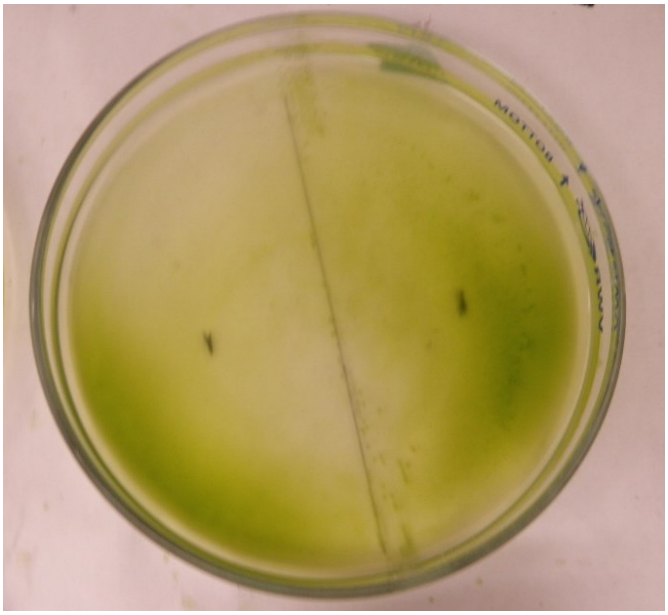
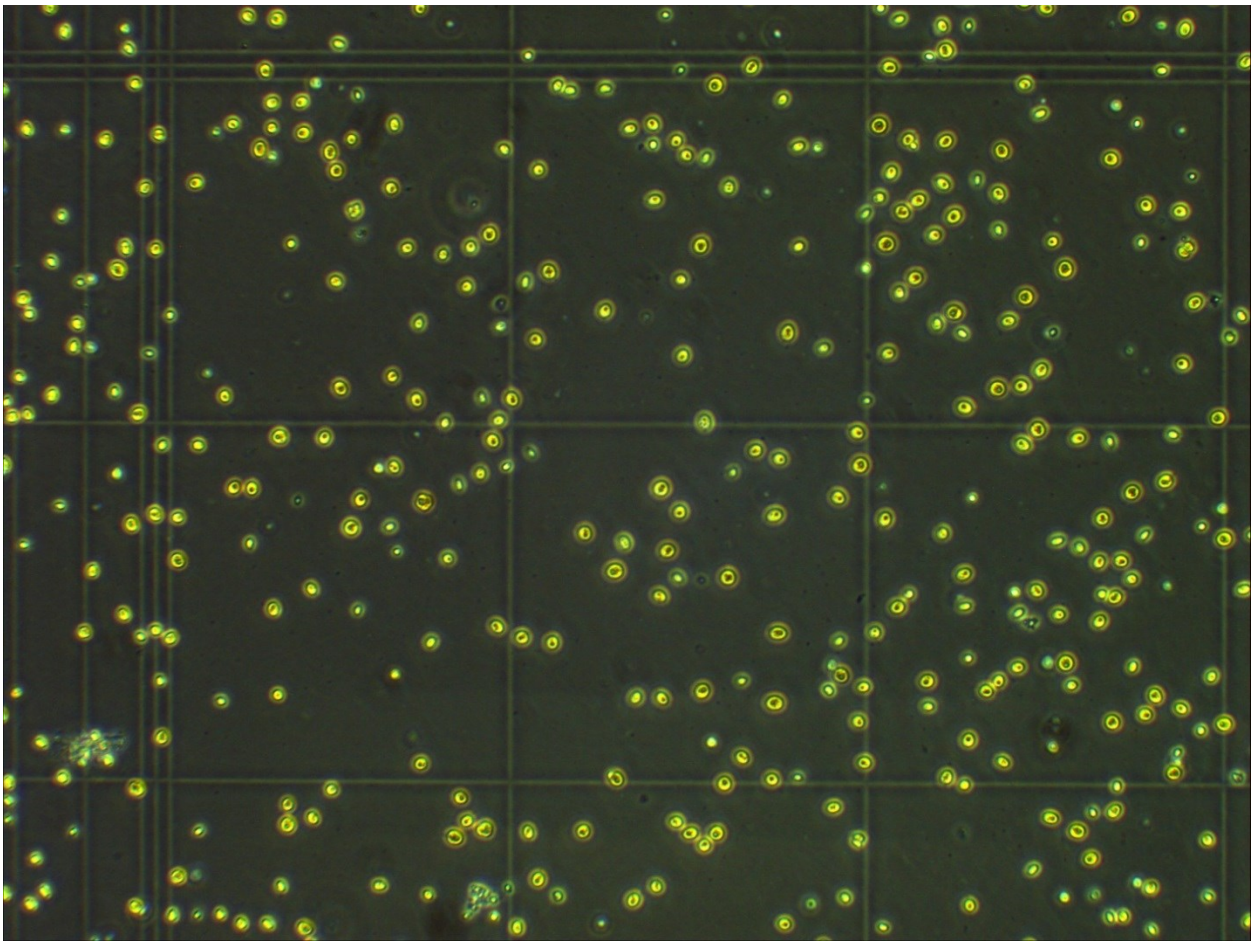


Figure A7: Photograph of immobilized *Chlamydomonas reinhardtii* cells in a haemocytometer chamber used to calibrate optical densities. Living cells are dark green. Dead cells (not counted) appear without complete cellular structure or vibrancy (Leboffe & Pierce 2005).



**Appendix 7:** DIFFUSION

Table A3: Comparisons of paired mean absorbancy values between the initial (dye added) and alternate (no dye) sides of Petri dishes at 12 hourly intervals. Degrees of freedom at 3 and 11 hours were reduced because I replaced one randomly chosen sample with a pure-media blank in order to calibrate the spectrophotometer for each of the two 96-well microplates evaluating absorbancy of the192 samples (16 samples × 12 hours). Bold lettering identifies statistically significant differences. Paired t-tests; two-tailed significance.

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| Hour | Side | Mean OD | Paired *T* | df | *P* |
|---|---|---|---|---|---|
| 0 | Initial | 0.059 | -9.23 | 7 | **<0.001** |
| | Alternate | 0.017 | | | |
| 1 | Initial | 0.048 | 9.45 | 7 | **<0.001** |
| | Alternate | 0.037 | | | |
| 2 | Initial | 0.056 | 7.86 | 7 | **<0.001** |
| | Alternate | 0.028 | | | |
| 3 | Initial | 0.052 | 9.65 | 6 | **<0.001** |
| | Alternate | 0.025 | | | |
| 4 | Initial | 0.044 | 2.34 | 7 | 0.054 |
| | Alternate | 0.040 | | | |
| 5 | Initial | 0.048 | 1.95 | 7 | 0.09 |
| | Alternate | 0.045 | | | |
| 6 | Initial | 0.044 | -1.79 | 7 | 0.12 |
| | Alternate | 0.048 | | | |
| 7 | Initial | 0.046 | -1.48 | 7 | 0.18 |
| | Alternate | 0.048 | | | |
| 8 | Initial | 0.044 | 0.04 | 7 | 0.97 |
| | Alternate | 0.044 | | | |
| 9 | Initial | 0.043 | 0.55 | 7 | 0.60 |
| | Alternate | 0.041 | | | |
| 10 | Initial | 0.043 | 0.10 | 7 | 0.928 |
| | Alternate | 0.043 | | | |
| 11 | Initial | 0.048 | 2.07 | 6 | 0.08 |
| | Alternate | 0.043 | | | |

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